

PATHOTYPES OF *COCHLIOBOLUS SATIVUS* (SPOT BLOTCH) ON BARLEY IN SYRIA

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SUMMARY

In order to study the Syrian pathotype diversity of *Cochliobolus sativus*, the causal agent of barley spot blotch disease, a survey was conducted using 31 isolates and 13 barley genotypes. Four pathotypes (pt 1-4) were identified based on the lesion form and infection response of the genotypes with mean disease rating from 1.76 to 7.46. Pt 1 exhibited low virulence on all used genotypes, pts 2 and 3 were moderately virulent and pt 4 was highly virulent. The two most common pathotypes were pt 3 (35%) and pt 4 (42%). The barley genotype AECS 71 was highly resistant to all pathotypes suggesting the existence of a general resistance mechanism. This genotype may be recommended as a possible donor in breeding programmes. The information obtained from this study should facilitate deploying effective resistance to *C. sativus* in barley.

Key words: Barley, *Hordeum vulgare*, virulence diversity, *Cochliobolus sativus*.

INTRODUCTION

Cochliobolus sativus (Ito and Kuribayashi) Drechs. ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc.) Shoem), the causal agent of spot blotch, is a common foliar pathogen of barley (*Hordeum vulgare* L.) worldwide, economically important because it can cause marked reduction in yield and quality of the crop (Mathre, 1982; Kiesling, 1985; Nutter *et al.*, 1985). Clark (1979) estimated barley yield losses from spot blotch to be 33% in susceptible Canadian cultivars. In Syria, van Leur (1991) reported a 40% yield loss in barley due to infection by *C. sativus*.

The development of barley genotypes resistant or tolerant to spot blotch is considered to be the most economic way for controlling this disease. Therefore, the pathotypes of *C. sativus* expressing different virulence on barley genotypes should be evaluated prior to any breeding steps.

With increased knowledge of the genetics of plant disease resistance, the virulent genotypes of pathotypes can be used to monitor changes in virulence gene frequencies in pathogen populations. Furthermore, potential pathotype numbers increase exponentially with the number of resistance genes used (Limpert and Muller, 1994). However, the selective pressure of pathogen isolates has led to the evolution of different resistance genes in barley (Valjavec-Gratian and Steffenson, 1997).

Under field conditions, it is impossible to estimate spot blotch resistance accurately, due to factors such as time and intensity of infection, level of inoculum, genotype and environmental interaction (Gilchrist *et al.*, 1995).

As spot blotch is a very important disease in Syria and no studies concerning the pathotype diversity of the *C. sativus* populations have been carried out there, we wished to gain information on its virulent pathotypes in different barley genotypes coming from widely dispersed areas. In this study we investigated, under controlled conditions, the pathotypes of *C. sativus* isolates collected from different region of Syria in 13 barley genotypes.

MATERIALS AND METHODS

Obtaining and maintaining isolates. During 2000 and 2001, isolates of *C. sativus* were obtained from barley leaves showing spot blotch symptoms in different regions of Syria. Leaf surfaces were sterilized in 10% sodium hypochlorite solution (NaOCl) for 3 min, soaked three times (5 min) in sterile distilled water, and dried between filter papers. The sterilized leaves were cut into small pieces 5 mm long and transferred to Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA). After incubation for 10 days, at 22 ± 1°C in the dark, 117 isolates of *C. sativus* were obtained.

Several barley cultivars were inoculated with these isolates and evaluated for host-parasite reaction. Thirty-one isolates showing differential reaction on specific cultivars were selected for evaluation on 13 barley genotypes. A suspension (made with conidia from 12-day-old *C. sativus* cultures) was adjusted to 2 × 10⁴ conidia per ml. The surfactant Tween 20 (polyoxyethylene-sor-

bitan monolaurate) was added (100 µl l⁻¹) to the conidial suspension to facilitate dispersion of the inoculum over the leaf surface.

Host plants. Thirteen barley genotypes were used (Table 1), chosen for their agricultural characteristics and diverse origins. Universal susceptible controls (cv. Arizona and WI2291 from USA and Australia, respectively) were included in the experiment.

The seeds were surface-sterilized with 5% sodium hypochlorite solution for 5 min and then washed three times in sterile distilled water. They were planted in plastic flats (60x40x8 cm) filled with sterilized peat-moss, and arranged in a randomized complete block design with three replicates. Each experimental unit consisted of two rows of 18 seedlings per genotype. A full replicate consisted of the plots of 13 genotypes inoculated with each of the 31 isolates; this full test was repeated three times. Flats were placed in a growth chamber at temperatures 22±1°C (day) and 17±1°C (night) with a day length of 12 h and a relative humidity of 80-90%. Seedlings were irrigated with Knop's nutrient solution (1 g NaNO₃; 0.25 g KNO₃; 0.25 g MgSO₄·7H₂O; 0.25 g KH₂PO₄; and 10 mg FeCl₃ per liter water).

Inoculation was performed at the two-leaf stage (GS) 11-12 (Zadoks *et al.*, 1974) by spraying uniformly 25 ml per flat of conidial suspension with a compressed air atomizer. After inoculation, plants were maintained in the dark at 95-100% relative humidity for the first 72 h after inoculation.

Disease assessment. Plant response to infection was scored 14 days after inoculation. The percentage of leaf area exhibiting disease symptoms for each genotype was determined using a numerical scale of 1 to 9 with 1 being the most resistant and 9 very susceptible (Fetch and Steffenson, 1999). Infection responses 1-3 were deemed resistant, 4-5 moderate, and 6 to 9 susceptible.

The STAT-ITCF program (Anonymous, 1988) was used to determine significant differences among means of disease ratings.

RESULTS AND DISCUSSION

The infection responses induced by the *C. sativus* isolates were clear and easy to score. The necrotic and chlorotic disease symptoms were always more severe in the very susceptible genotypes. Four pathotypes (pts 1-4) were identified based on the infection response (lesion form and virulence patterns) of each genotype. Pt 1 induced small round to oblong dark brown necrotic lesions. Pt 2 induced elongated light brown necrotic lesions with expanding areas of chlorosis. Pt 3 induced light brown lesions with whitish gray centers and chlorotic margins, and Pt 4 induced soiled dark brown necrotic lesions with expanding chlorosis (the 'classic' spot blotch lesion) in highly compatible interactions. The two commonest pathotypes were pt 3 (35%) and pt 4 (42%) (Table 1). The other two pathotypes accounted for the remaining seven isolates. Pt 1, however, is of no

Table 1. Pathotypes numbers and mean disease rating of 31 Syrian isolates of *C. sativus* on 13 barley cultivars ^a.

Group No.			1	2	3	4
Pathotype No.			pt1	pt2	pt3	pt4
No. of isolate			2	5	11	13
Genotypes	Row type	Origin				
AECS 83	2	Syria	R	S	S	S
AECS 76	6	Syria	R	M	R	S
AECS 71	6	Syria	R	R	R	R
Arabi Abiad	2	Syria	R	R	M	S
Furat-2	6	Syria	R	M	M	S
Arizona	6	USA	R	S	S	S
Arrivate	6	USA	R	M	M	S
CI-5791	2	Ethiopia	R	R	R	M
Golf	2	England	R	S	S	S
Thibaut	6	France	R	R	R	M
Selina	2	France	R	S	S	S
WI 2291	2	Australia	R	S	S	S
Smash	6	Belgium	R	S	S	S
Mean disease rating ^b			1.76 d ^c	3.06 c	4.48 b	7.46 a

^a R: Resistant, M: moderate and S: Susceptible according to a scale 1 to 9 (Fetch and Steffenson, 1999).

^b Values are the average of the disease rating of 13 barley genotypes replicated three times, calculated on the basis of 1-9 scale (see the text).

^c Means followed by different letters are significantly different ($P < 0.001$) according to Newman-Keuls test.

importance in screening breeder's material due to its lack of virulence. The pathotypes, genotypes used and isolates are shown in Table 1.

Analysis of variance showed highly significant differences ($P < 0.001$) for virulence levels among the four separated pathotypes. The mean disease rating was 7.46 in pt 4. Pt 3 had an intermediate virulence with mean disease rating of 4.88. Pt 1 was composed of avirulent isolates with mean disease rating of 1.76 (Table 1).

This study, conducted under controlled conditions, discriminated four pathotypes of *C. sativus* based on the infection response of barley genotypes. These genotypes were effective for characterizing virulence because they all gave clear responses to the pathogen isolates studied (Table 1). The inoculation protocol used in our study consistently produced sufficient numbers of well-separated lesions for assessing infection responses on barley plants, and similar results were obtained in the three replications of the experiment.

Certain barley cultivars (i.e., AECS 71 and CI-5791) showed a high level of resistance (Table 1). This confirms the importance of the Middle-East as a source of resistant genotypes to spot blotch. On the other hand, American 'Arizona' and Australian 'WI2291' genotypes were always susceptible. These results are in agreement with those of van Leur (1991), Wilcoxon *et al.* (1990) and Steffenson *et al.* (1996). The genotype AECS71 was identified as being extremely resistant to all isolates used. The presence of cultivars highly susceptible to this pathogen suggests that natural selection pressure is not a reliable source of resistance since it does not occur in a sufficiently uniform manner (Bailey *et al.*, 1988).

The variation in the virulence of *C. sativus* observed in this study is in agreement with the results of Fetch and Steffenson (1994) and Meldrum *et al.* (2000). This variation can be attributed to the interactions of cultivars and it is assumed that several virulence genes are operating in the pathosystem (Hetzler *et al.*, 1990). The specificity of pathosystems and the different types of resistance have been defined by Van derPlank (1984).

Although we tested only 31 isolates and 13 barley genotypes, *C. sativus* does not appear as variable as the two other common foliar pathogens of barley, *Rhynchosporium secalis* and *Pyrenophora teres* f. *teres*. Tekauz (1990, 1991) identified 28 *R. secalis* pathotypes out of 51 isolates, and 45 *P. teres* pathotypes out of 182 isolates using only 9 barley genotypes. The low variability of *C. sativus* pathotypes found in our study agrees with that of Valjavec-Gratian and Steffenson (1997) who found three pathotypes of *C. sativus* among 22 isolates tested on three barley genotypes in North Dakota. The classification of different pathotypes of *C. sativus* facilitated the molecular mapping of a spot blotch resistance locus, and selection of resistant lines in the field (Steffenson *et al.*, 1996).

Our results indicate the need to monitor the Syrian pathotypes of *C. sativus*, for detecting sources of infection, predicting the spread of disease across locations,

and studying local extension and recolonization. Moreover, knowledge of pathotype virulence levels, obtained in this study, may aid in plant breeding programmes, through use of the most virulent pathotypes to select new promising resistant lines. The barley genotype AECS71, most resistant to spot blotch, could be considered as a possible donor in further breeding programmes.

ACKNOWLEDGMENTS

The authors thank the Director General of the Atomic Energy Commission of Syria and the Head of the Biotechnology Department for their support. They also thank Dr. N. MirAli for his critical reading of the manuscript.

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Received 12 February 2003

Accepted 18 July 2003